CH --20. The fusion protein of claim 18 wherein said label is a lanthanide.--

REMARKS

In response to the Restriction Requirement, nonelected claims 11-16 have been canceled. Applicant reserves the right to refile these canceled claims as part of a divisional application.

Claims 3 and 8 have been amended to refer to SEQ ID NOs.

Claim 4 has been amended to correct a typographical error.

New claims 18-20 have been added. These are discussed below.

A new Abstract has been submitted. Other than the first line, the new Abstract is identical to the originally submitted Abstract except that it has been retyped as a single paragraph rather than as two paragraphs. It is urged that the substitute Abstract includes no new matter.

The 35 U.S.C. § 112, Second Paragraph, Rejection

Claims 1-10 and 17 were rejected under 35 U.S.C. § 112, second paragraph as being indefinite. Claim 1 has been rewritten to use proper Markush language. Although no rejection was made of claim 7 for being indefinite, it too has been amended in a manner similar to the amendment of claim 1. In view of the amendment it is urged that the claims are no longer indefinite and it is requested that the rejection under 35 U.S.C. § 112, second paragraph, be withdrawn.

The 35 U.S.C. § 103(a) Rejections

Claims 1-5, 7-10 and 17 were rejected under 35 U.S.C. § 103(a) as being obvious over Rogers et al. in view of Hummel et al., Verge et al., Rabin et al, Borg et al., Berg et al. and Wiest-Ladenburger et al.

Although the cited references teach recombitopes in general and that recombitopes can be used to assay for diabetes, there are nonobvious differences between the invention and the cited prior art. It is pointed out that no cited prior art reference combined all three proteins (IA2,

GAD65, and preproinsulin) into a single molecule. Furthermore, none of the prior art references teaches preproinsulin as part of a recombitope. The combination of preproinsulin plus one of GAD65 or IA2 or the combination of the three proteins forms a three dimensional structure with exposed epitopes not predictable from the cited prior art and therefore it would have been unpredictable whether any appropriate epitopes to react with antibodies in persons at risk for diabetes would be exposed and accessible to the antibodies. Therefore the ability of the claimed compound could not have been predicted. Furthermore, the recombitopes of the invention are large. The fusion protein of claim 3 requires approximately 800 amino acid residues. This is a large protein of approximately at least 88000 kDa. The exact folding and three dimensional structure of this protein could not be determined from the cited prior art. Until such a protein was made and tested there would have been an unknown chance that not all of the protein "fragments" would have an accessible epitope to react with antibodies in blood samples. If one or more of the "fragments" did not have an active epitope that could be recognized then the fusion protein would not be so useful as it in fact is. As noted, this could not have been known *a priori* even in view of the cited references. The Rogers et al. reference discusses preferred lengths in column 10, lines 48-62. Although those lines state that peptides can comprise as many amino acid residues as desired and most preferably contain at least about 40 residues, it continues on to state that a region of a recombitope preferably comprises up to 45 amino acid residues and most preferably up to 30 amino acid residues in length, as increases in length of a region of a recombitope peptide may result in difficulty in peptide synthesis as well as retention of an undesirable property.

The informativity of the assay set forth in the application is based on presence of all those epitopes on the autoantigen (fusion protein) which cause the individual to induce autoantibody formation. It is well known that these autoantibodies are directed to very different parts of, e.g., GAD or IA2, e.g., there are antibodies recognizing the N-terminal domain of IA2 as well as such recognizing different epitopes at the C-terminal half. Short peptide recombitopes as suggested by Rogers will not comprise all of these epitopes and are thus not necessarily as informative in a single assay, as there is no information available on whether the presence of autoantibodies against a specific epitope would be of more predictive or informative value than autoantibodies against other epitopes. Moreover, there probably is no single pathogenic epitope as IDDM

patients show the presence of autoantibodies directed to different regions within a protein and even to different proteins, most often Gad65 and IA2 or both.

The recombitope or fusion protein of claim 3 comprises at least 209 amino acid residues of IA2, 484 residues of GAD65, and 110 residues of preproinsulin. These are each much longer than the preferred and most preferred lengths taught by the Rogers et al. patent which in fact taught against making the epitope fragments too long. Because the Rogers et al. reference teaches away from such long pieces, it is apparent that the utility of the claimed fusion protein could not have been determined until it was actually tried. Furthermore, the preferred suggested lengths of the Rogers et al. patent would have taught one to avoid such long lengths, thereby teaching away from the invention.

Claim 3 also may be limited to specific portions of IA2 and GAD65. The prior art did not teach whether these portions of the proteins would be recognized by antibodies present in persons with diabetes. For example, the Wiest-Ladenburger reference in the last two full sentences on page 565 states that the region 603-979 of IA2 was known to be the region identified as the target of ICAs (islet cell antibodies). This alone is not enough to determine whether the smaller region of 771-979 would alone also be recognized by such antibodies in the absence of the 603-770 region of the protein. Similarly, the cited prior art does not teach using only the 102-585 region of GAD. The Wiest-Ladenburger reference used the complete GAD molecule. The Rabin et al. patent gave one example and again the whole GAD protein was used (see GAD purification beginning at column 9, line 37). The Hummel et al. reference simply states that a recombinant GAD was used without mentioning what size and therefore it was likely the complete molecule. The Verge et al. reference teaches use of a recombinant GAD of 65 kD (see first paragraph in right hand column on page 380). The 102-585 region of GAD used in the invention is only approximately 53 kD, thereby missing regions of GAD which were used in the prior art references and thereby leaving doubt as to whether the necessary epitope is present. The Borg et al. reference also used the complete 65 kD GAD (see second full paragraph of right hand column on page 2359).

Newly added claims 18-20 are discussed here in view of the obviousness rejection which was set forth. These claims are drawn to a labeled fusion protein comprising epitopes of at least two of IA2, GAD65 and preproinsulin. A labeled fusion protein can be used as shown in Figure 5 of the application. The labeled fusion protein is used as the probe to measure the sum of the

bound antibody in the assay. None of the cited prior art references used a fusion protein or recombitope in a labeled fashion as the probe. When IA2, GAD or preproinsulin was labeled and used as a probe in the cited prior art references it was always as a separate, discrete molecule, not as part of a fusion protein. There is no suggestion or motivation in the cited references to label a fusion protein. The prior art either used each protein singly to measure the level of the particular corresponding antibody or it used a mixture of nonfusion proteins to measure the sum of the antibodies, but never was the fusion protein labeled and used as a probe in the cited references. The use of a single molecule as a fusion protein simplifies the assay yielding faster and more economical results. This is simply one more reason, in addition to those discussed above, that claims 18-20 are nonobvious in view of the cited prior art.

Claims 1-10 and 17 were rejected under 35 U.S.C. 103(a) as obvious over Rogers et al., in view of Hummel et al., Verge et al., Rabin et al., Borg et al., Berg et al., and Wiest-Ladenburger et al. and further in view of WO 94/07464. The only difference between this rejection and that described above is the inclusion of WO 94/07464 as a teaching of biotin or streptavidin as part of the fusion protein which is a limitation of claim 6. The added reference does not teach the shortcomings of the prior art discussed above, e.g., it does not teach the combination of IA2, GAD and preproinsulin in a single molecule, does not teach fusion protein of large size, does not teach the use of the specific fragments of the proteins, and does not teach a labeled fusion protein. Consequently the same arguments presented above still hold for this latter 103(a) rejection.

Because of the differences between the claims and the cited prior art, i.e., the fact that such recombitopes had never been made, that the claimed recombitopes can be very large, and that specific protein fragments were not clearly known to include appropriate epitopes, the prior art at best leads to an obvious to try analysis.

In view of the arguments set forth above, it is requested that the rejections of the claims under 35 U.S.C. 103(a) be withdrawn.

In view of the amendments and the above arguments, it is submitted that the present claims satisfy the provisions of the patent statutes and are patentable over the prior art. Reconsideration of this application and early notice of allowance are requested.

Respectfully submitted,

Stephen A. Saxe

Stephen A. Saxe
Registration No. 38,609

ROTHWELL, FIGG, ERNST & KURZ, P.C.
555 Thirteenth St., N.W., Suite 701 East Tower
Washington, DC 20004
Telephone: (202) 624-1589
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